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I hereby certify that this paper is being deposited on this date with the U. S. Postal Service as first class mail addressed to the Commissioner of Patents and Trademarks, Box 1450, Alexandria, VA 22313-1450.

Ying-Zi Yang — 7/22/05 (Date)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR § 1.132

Sir:

I, Margaret Karow , declare as follows:

1. I hold a Ph.D. in Cellular, Viral, and Molecular Biiology from the University of Utah (copy of *curriculum vitae* attached). I presently hold the position of Vice President, Traps, Therapeutic Antibody and Small Molecule Technologies at, and have an interest in Regeneron Pharmaceuticals, Inc., having its principal place of business at 777 Old Saw Mill River Road, Tarrytown, New York 10591. I am familiar with Regeneron's angiopoietin constructs and with the above-identified patent application. I conducted the experiments described below in October of 1998 which compared the *in vitro* properties of different Ang1 and Ang2 constructs.

2. Competition Experiments. I conducted competition assays to obtain IC50 values as described in Davis et al. 2003 Nature Structural Biology 10:38-44, p. 43. Briefly, competition experiments were performed with Ang1*, Ang2, TL1-CF, Ang1FD-Fc-FD, Ang2FD-Fc-FD and Ang1-FD-FD-Fc competing with europium labeled Ang1* for binding to Tie2-Fc. Ang1* is a chimeric form of Ang1 in which the N domain of Ang1 is replaced with the N domain of Ang2, and the cysteine residue at 245 of Ang1 is replaced by serine (described in U.S.S.N. 09/868,677 at page 23, lines 28-30) and TL1-CF is the coil-coil domain and fibrinogen domain of Ang1. The results showed that the "bow" configuration of Ang1FD-Fc-FD exhibited an IC50 similar to that of Ang1* (2.5 vs. 4.5 nM) and significantly better than a molecule having a "tower" configuration of Ang1FD-FD-Fc (35 nM). IC50s molarities were estimated using the molecular weight of the monomer of each of these molecules.

TABLE 1. Competition with 1 nM Ang1* for TIE2-Fc Binding

Construct	**Monomer Molecular Weight**	**Structure**	**Estimated IC50**
Ang1*	55 Kda	tetrameric and higher	2.5 nM
Ang2	55 Kda	tetrameric and higher	10 nM
TL1-CF	50 Kda	divalent dimeri only	100 nM
Ang1FD-Fc-FD	76 Kda	tetravalent dimer only	4.5 nM
Ang2FD-Fc-FD	76 Kda	tetravalent dimer only	65 nM
Ang1-FD-FD-Fc	76 Kda	tetravalent dimer only	35 nM

3. Pharmacokinetics. I have reviewed the results of earlier experiments conducted and recorded by Regeneron to determine the pharmacokinetic profiles of Ang1* and Ang1FD-Fc-FD. The experiments were initiated December 27, 2000. Basically, each construct was tested by administering 25 mg/kg to each of 3 mice using either subcutaneous or intravenous routes. Serum samples were taken at sequential time points for 7 days and the samples were analyzed by ELISA to determine protein levels (Table 2).

Generally, the Cmax measurement reflects how much of the angiopoietin

construct quickly enters the blood, the T1/2 measurement reflects how long the construct remains in the blood, and the area under the curve reflects both parameters and provides a measure of the total exposure of the animal to the construct. The results show that the "bow" Ang1 construct had significantly improved pharmacokinetic profile relative to the Ang1* construct.

TABLE 2

	Cmax (ug/ml)	**$T_{1/2}$ (hr)**	**Area under the Curve (ug·hr/ml)**
Intravenous injection			
Ang1*	4.55 ± 0.35	3.80 ± 0.07	14.42 ± 0.41
Ang1-FD-Fc-FD	33.27 ± 7.95	9.85 ± 0.53	173.34 ± 20.67
Subcutaneous injection			
Ang1*	1.45 ± 0.66	9.25 ± 2.88	5.64 ± 1.86
Ang1-FD-Fc-FD	26.24 ±7.04	18.31 ± 2.5	243.58 ± 51.77

4. I hereby declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that I make these statements with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

7/21/05
Date

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Personal Information

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Employment Experience

Jan 2004-Present

Vice President, Traps, Therapeutic Antibody and Small Molecule Technologies at Regeneron Pharmaceuticals

- Identification of drug targets that have strong support for being validated, work with the legal department to determine what are the Intellectual Property issues surrounding the target, and develop a program around the target. Once initiated, I manage the design, testing, and validation of Traps. This includes the development and qualification of biochemical, cell based assays, acute and disease animal models.
- Team leader for the IL-18 Trap, which is in pre-clinical development and is schedule for phase I testing in Q106.
- Oversee assay development for high throughput assays with Proctor and Gamble, and cell-based and biochemical assays for pre-clinical and clinical therapeutics.
- Direct the phenotyping of Velocigene knockout mice for immunologically related genes; including ex vivo assays, acute models, and disease models, as well as manage outside collaborations for immune-based phenotyping.
- Supervise in-house monoclonal antibody facility, which produces antibodies and assays for validation of targets, pre-clinical development and clinical programs.
- Co-supervise the development and characterization of Velocimmune mice, which produces human antibodies from the mouse endogenous loci.
- Supervise the development and characterization of mice producing novel antibodies and mice with human MHC class II haplotypes
- Group size: Direct reports 7 PhD Scientist and 2 Senior Research Associates; Indirect reports, 10 Research Associates

Jan., 2001-Jan. 2004.

Director, Therapeutic Antibody and Small Molecule Technologies at Regeneron Pharmaceuticals

- Lead Scientist for collaboration with Medarex to produce therapeutic human antibodies. Oversaw all aspects of the program including identification of validated targets, design of target-specific assays including biochemical, cell based assays, acute and disease animal models.
- Assay development for high throughput assays with Proctor and Gamble, as well as cell-based assays for pre-clinical and clinical therapeutics.
- Phenotyping of Velocigene knockout mice for immunologically related genes; including ex vivo assays, acute models, and disease models.
- Supervise in-house monoclonal antibody facility, which produces antibodies and assays for validation of targets, pre-clinical development and clinical programs.
- Co-supervised the development and characterization of Velocimmune mice, which produce human antibodies from the endogenous loci.
- Group size; Direct reports, 4 PhD Scientists and 1 Senior Research Associate; Indirect reports, 6 Research Associates

Jan., 2000-Jan, 2001.

Associate Director, Small Molecule Discovery Program at Regeneron Pharmaceuticals

- Supervised development and validation of high throughput screens and secondary assays, for kinases, phosphatases, and GPCRs, for Regeneron's collaboration with Proctor and Gamble.
- Supervised the development and validation of cell-based assays for Traps in pre-clinical and clinical stages
- Group size; Direct reports, 2 PhD Scientists and 2 Research Associates; Indirect reports 2 Research Associates

Jan., 1999-Jan., 2000.

Senior Staff Scientist, Small Molecule Discovery Program at Regeneron Pharmaceuticals

- High throughput screen development and secondary assays development of protein-protein interaction, kinase, and GPCR screens for Regeneron's collaboration with Proctor and Gamble.
- Group size; Direct Reports, 2 PhD Scientist and 2 Research Associates

Jan., 1998-Jan., 1999.

Staff Scientist, Small Molecule Discovery Program at Regeneron Pharmaceuticals

- High throughput screen development, secondary assays development, and acute animal model development for protein-protein interaction, kinase, and phosphatase targets for Regeneron's collaborations with Proctor and Gamble and Pharmacopeia.
- Group size; Direct Reports, 1 PhD Scientist and 2 Research Associates

Jan., 1997-Jan., 1998.

Scientist, Small Molecule Discovery Program at Regeneron Pharmaceuticals

- ❖ Responsibility for *E.coli* expression and purification of proteins, their characterization, and assay development for protein-protein interaction, kinase and phosphatase screens performed in collaboration with Pharmacopeia.
- ❖ Group size; Direct Reports, 1 Research Associate

Nov., 1995-Jan., 1997.
Scientist, Protein Sciences Group at Regeneron Pharmaceuticals

- ❖ Developed Pichia Pastoris expression system for high yield production of proteins with fibrinogen domains.

1992-1995.
Post-doctoral work with Dr. Patrick J. Piggot at Temple University

- ❖ Molecular and genetic research to identify factors required for the activation of sigma factors during the sporulation of *Bacillus subtilis*; isolation and characterization of the *spoIIR gene.*

1988-1992.
Graduate student with Dr. Costa Georgopoulos at the University of Utah

- ❖ Research to determine the function of the *Escherichia coli high* temperature requirement gene *htrB* and its related suppressors, *msbA* and *msbB*.

1987-1988.
Graduate student with Dr. Tulle Hazelrigg at the University of Utah

- ❖ The isolation of a UV induced deletion mutation, and cloning of the *Drosophila Melanogaster exuperantia gene.*

1984-1987.
Technician with Dr. Kathleen Danna, at the, University of Colorado

- ❖ In vivo and in vitro studies of the processing of nascent SV40 late transcripts.

Education

Ph.D. from the University of Utah in the Department of Cellular, Viral, and Molecular Biology. Graduated March, 1992.

B.A. from the Department of Molecular, Cellular, and Developmental Biology at the University of Colorado at Boulder. Graduated with Distinction, May, 1982.

Publications

Lund JM, Alexopoulou L, Sato A, Karow M, Adams NC, Gale NW, Iwasaki A, Flavell RA. Recognition of single-stranded RNA viruses by Toll-like receptor 7. (2004) Proc Natl Acad Sci U S A. 101:5598-5603

Davis S, Papadopoulos N, Aldrich TH, Maisonpierre PC, Huang T, Kovac L, Xu A, Leidich R, Radziejewska E, Rafique A, Goldberg J, Jain V, Bailey K, Karow M, Fandl J, Samuelsson SJ, Ioffe E, Rudge JS, Daly TJ, Radziejewski C, Yancopoulos GD. Angiopoietins have distinct modular domains essential for receptor binding, dimerization and superclustering. (2003) Nat Struct Biol.;10:38-44

Karow ML, Rogers EJ, Lovett PS, Piggot PJ. Suppression of TGA mutations in the Bacillus subtilis *spoIIR* gene by *prfB* mutations. (1998) J Bacteriol.;180:4166-4170.

Carpenter LR, Farruggella TJ, Symes A, Karow ML, Yancopoulos GD, Stahl N. Enhancing leptin response by preventing SH2-containing phosphatase 2 interaction with Ob receptor. (1998) Proc Natl Acad Sci U S A.;95:6061-6066.

Zhang L, Higgins ML, Piggot PJ, Karow ML. Analysis of the role of prespore gene expression in the compartmentalization of mother cell-specific gene expression during sporulation of *Bacillus subtilis*. (1996) J Bacteriol.;178:2813-2817.

Karow ML, Piggot PJ. Construction of *gusA* transcriptional fusion vectors for *Bacillus subtilis* and their utilization for studies of spore formation. (1995) Gene ;163:69-74.

Karow ML, Glaser P, Piggot PJ. Identification of a gene, *spoIIR*, that links the activation of sigma E to the transcriptional activity of sigma F during sporulation in *Bacillus subtilis*. (1995) Proc Natl Acad Sci U S A.; 92:2012-2016.

Karow M, Georgopoulos C. The essential *Escherichia coli msbA* gene, a multicopy suppressor of null mutations in the *htrB* gene, is related to the universally conserved family of ATP-dependent translocators. (1993) Mol Microbiol.;7:69-79.

Karow M, Fayet O, Georgopoulos C. The lethal phenotype caused by null mutations in the *Escherichia coli htrB* gene is suppressed by mutations in the *accBC* operon, encoding two subunits of acetyl coenzyme A carboxylase. (1992) J Bacteriol.;174:7407-7418.

Karow M, Georgopoulos C. Isolation and characterization of the Escherichia coli *msbB* gene, a multicopy suppressor of null mutations in the high-temperature requirement gene *htrB*. (1992) J Bacteriol.;174:702-710

Karow M, Georgopoulos C. Sequencing, mutational analysis, and transcriptional regulation of the *Escherichia coli htrB* gene. (1991) Mol Microbiol.;5:2285-2292.

Karow M, Raina S, Georgopoulos C, Fayet O. Complex phenotypes of null mutations in the htr genes, whose products are essential for *Escherichia coli* growth at elevated temperatures. (1991) Res Microbiol.;142:289-294.

Karow M, Fayet O, Cegielska A, Ziegelhoffer T, Georgopoulos C. Isolation and characterization of the *Escherichia coli htrB* gene, whose product is essential for bacterial viability above 33 degrees C in rich media. (1991) J Bacteriol.;173:741-50.

Hazelrigg T, Watkins WS, Marcey D, Tu C, Karow M, Lin XR. The *exuperantia* gene is required for *Drosophila* spermatogenesis as well as anteroposterior polarity of the developing oocyte, and encodes overlapping sex-specific transcripts. (1990) Genetics ;126:607-617.

Salmon ED, Leslie RJ, Saxton WM, Karow ML, McIntosh JR. Spindle microtubule dynamics in sea urchin embryos: analysis using a fluorescein-labeled tubulin and measurements of fluorescence redistribution after laser photobleaching. (1984) J Cell Biol.;99:2165-2174.

Salmon ED, Saxton WM, Leslie RJ, Karow ML, McIntosh JR. Diffusion coefficient of fluorescein-labeled tubulin in the cytoplasm of embryonic cells of a sea urchin: video image analysis of fluorescence redistribution after photobleaching. (1984) J Cell Biol.;99:2157-2164.

Awards

Individual National Research Service Award from the U.S. Public Health Service, the Allergy and Infectious Diseases division of the NIH. Three Year award Starting July, 1994..

The David R. and Isabelle E. Atherton Scholarship award for Ph.D. candidates in the University of Utah School of Medicine. 1991

The 1992 James W. Prahl Memorial Award for the outstanding graduate student in the University of Utah School of Medicine.